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Annotated Sequence Record

Characterization of complete sequences of RNA 1 and RNA 2 of citrus variegation virus

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Introduction

Citrus variegation virus (CVV), a member of the genus *Ilarvirus*, was originally isolated from citrus but was reported to infect many herbaceous plants under greenhouse conditions [3]. The virus particles are approximately 30 nm in diameter [1] and are composed of coat protein (CP) with a Mr of 26 kDa, and five RNA species designated as RNAs 1, 2, 3, 4 and 4a, respectively [6]. A very close serological relationship was found between CVV and citrus leaf rugose virus (CLRV), a member of *Ilarvirus* subgroup 2 [4, 5]. Both putative translation products of the complete sequence of CVV RNA 3 (GenBank accession U17389) and of the partial ORF from the partial sequence of RNA 2 (GenBank accession U93605) were closely related to elm mottle virus (EMoV), another member of subgroup 2 [9–11]. These two pieces of evidence suggested CVV be placed into subgroup 2 of the genus *Ilarvirus*. To date, the sequence for CVV RNA 3 (2309 nt) has been completed [8], but only partial sequences for CVV RNAs 1 (GenBank accession U93604) and 2 are available [10]. This report presents the complete sequences of CVV RNA 1 and RNA 2. Phylogenetic analysis of the putative replicase and polymerase proteins of CVV and the corresponding proteins of other ilarviruses supports placement of CVV as a member of *Ilarvirus* subgroup 2 [9–11].

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Provenance of the virus material

CVV was identified originally from grapefruit (*Citrus paradisi* Macf.) in Florida in 1960 [1] and graft transmitted to Eureka lemon (*C. limon* [L.] Burm. f.) at the USHRL, Orlando, Florida, with subsequent sap transfer to *Phaseolus vulgaris* (var. Red Kidney Bean), followed by local lesion transfer from red kidney bean to citrus, producing isolate CVV1. CVV1 has been maintained at the USHRL in Orlando and Fort Pierce with periodic graft transmissions to citrus hosts for more than 40 years. The sequence described here was obtained from virus propagated in *Nicotiana benthamiana*, which had been inoculated with sap from the current citrus host Rough lemon (*C. jambhiri* Lush). For cloning and sequencing, CVV1 was inoculated

to *N. benthamiana* and total RNA was extracted from *N. benthamiana* leaf tissue with an RNeasy kit (Qiagen, CA) according to manufacturer's instructions. The full-length sequences of RNAs 1, 2 and 4 of this virus were determined by standard RT-PCR and RACE techniques [7]. Phylogenetic comparisons of the sequences were completed with DNAMAN version 4.0 (Lynnon BioSoft, Vaudreuil, Quebec, Canada) using the neighbor-joining method.

Sequence properties

Figure 1 shows the genomic organization of isolate CVV1. CVV RNA 1 (GenBank accession EF584664) is 3433 nt long and contains a single open reading frame (ORF), which codes for the p1 protein, a putative translation product of 1076 amino acids (aa) with a Mr of 121,117 Da. The N-methyl-transferase-like and helicase-like signatures are located within aa 88–180 and 770–1023 in the p1 protein, respectively.

CVV RNA 2 (GenBank accession EF584665) is 2914 nt long and is bicistronic, with two overlapping ORFs located at positions 73–2445 and 2126–2722, respectively. The 5′-proximal ORF encodes the p2 protein, a putative translation product of 790 aa with a calculated Mr of 90,477 Da. The polymerase signature is located within aa 432–525 of this protein. The 3′-proximal ORF encodes a putative protein of 198 aa with a calculated Mr of 22,101 Da, which is similar to the 2b protein encoded by other ilarviruses [11].

The nucleotide sequences of the 5′ untranslated regions (UTRs) of both RNA 1 and RNA 2 are 72 nt long and are identical except for one substitution at position 62. The 5′UTR of RNA 3 is much larger (354 nt) and has no sequence similarity with those

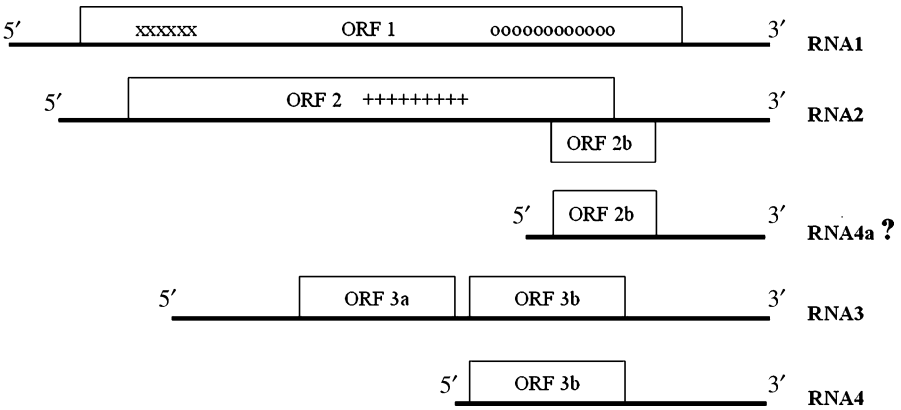
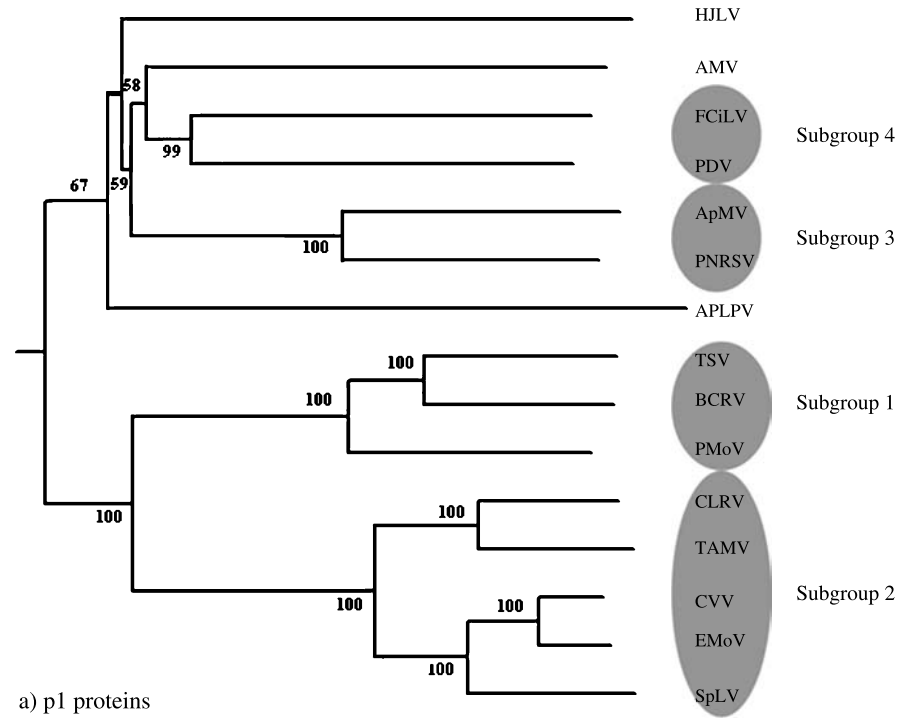
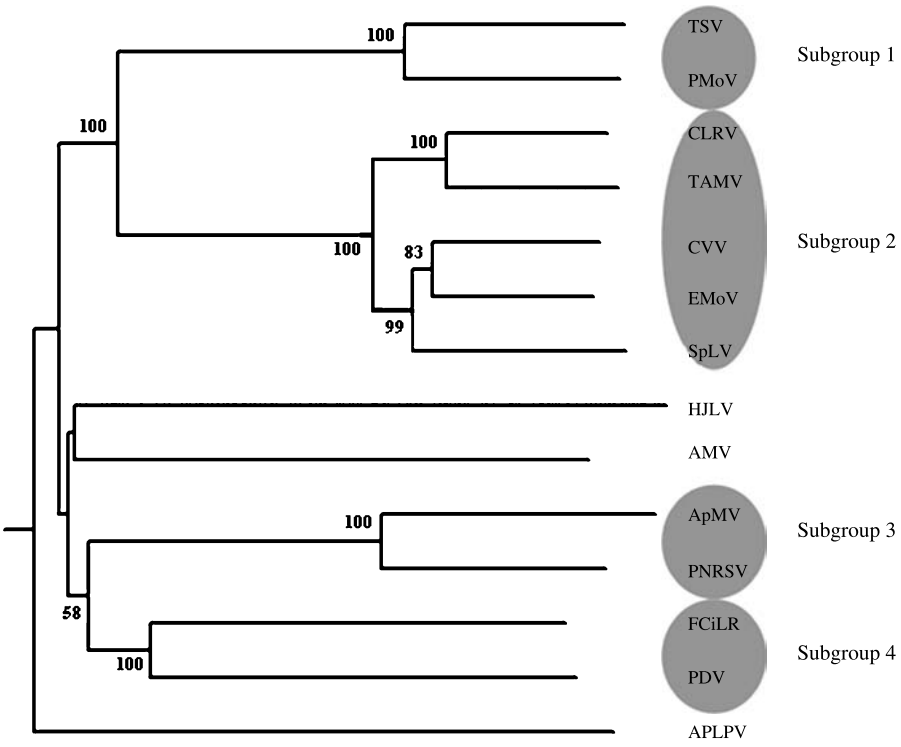


Fig. 1. Schematic representation of the genomic organization of CVV. The open reading frames are shown as boxes, and the N-methyl transferase, helicase, and polymerase signatures are indicated by xxxx, oooo and ++++, respectively. The "?" refers to the proposed identity of RNA 4a observed by Gonsalves and Garnsey [6]

Fig. 2. Phylogenetic trees derived from alignment of the aa sequences of **a**) the p1 protein and **b**) the p2 protein of CVV with the homologous proteins from the indicated ilarviruses and from the related alfalfa mosaic virus (AMV). Genbank accession numbers for RNAs 1 and 2 of the indicated viruses are respectively: AMV – L00163, X01572; apple mosaic virus (ApMV) – AF174584, AF174585; American plum line pattern virus (APLPV) – AF235033, AF235165; blackberry chlorotic ringspot virus (BCRV) – DQ091193; CLRV – NC_003548, NC_003547; EMoV – NC_003569, NC_003568; Fragaria chiloensis latent virus (FCiLV) – NC_006566, NC_006567; Humulus japonicus latent virus (HJLV) – AY500236, AY500237; PMoV – AY496068, AY496069; prune dwarf virus (PDV) – U57648, AF277662; PNRSV – AF278534, AF278535; SpLV – NC_003808, NC_003809; Tulare apple mosaic virus (TAMV) – NC_003833, NC_003834; and TSV – U80934, U75538. The trees were generated by the neighbor-joining method using DNAMAN and were bootstrapped using 1000 replications. The subgroups within the genus *Ilarvirus* are labeled as such and are indicated by gray circles



a) p1 proteins



b) p2 proteins

of RNAs 1 and 2, although all three genomic RNAs have the same initial five bases (GTATT).

The sizes of the 3′ UTRs of RNAs 1, 2 and 3 are dissimilar at 130, 192 and 327 nt, respectively. There is variability in sequence identity as well. There is 81% identity between the terminal 130 nt of RNAs 1 and 2 or between RNAs 1 and 3, but 95% identity between the terminal 192 nt of RNA 2 and RNA 3.

RNA 4 is 1033 nt long and is the subgenomic RNA for the CP ORF, corresponding to positions 1277–2309 of RNA 3. Previous analysis of encapsidated CVV RNAs indicated the presence of a small RNA which was designated RNA 4a [6]. RNA 4a is smaller than RNA 4 and is not characterized yet. Cucumoviruses and some ilarviruses in subgroup 1 and subgroup 2 produce a subgenomic RNA from RNA 2 for expression of the 2b protein [2, 12]. CVV RNA 4a may be a subgenomic RNA generated from CVV RNA 2 to express the 2b protein. The putative CVV 2b protein shares ∼60% identity with the homologous proteins of spinach latent virus (SpLV) and EMoV, both of subgroup 2, but only ∼15% identity with tobacco streak virus (TSV) and Parietaria mottle virus (PMoV), two members of subgroup 1 [9–11].

The genomic organization (Fig. 1) and the deduced sizes of the putative translation products of CVV are in good agreement with known sizes of some typical ilarviruses. Neighbor-joining analysis of the amino acid sequences of both the p1 and p2 proteins of CVV (Fig. 2) strongly supports placement of CVV as a member of subgroup 2 of the genus *Ilarvirus* [9–11]. Combined with the phylogenetic analysis of CVV CP [9], this result also indicates that, just like the other characterized viruses in subgroup 2, CVV genomic RNAs 1, 2 and 3 have apparently evolved together rather than having different phylogenetic origins.

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